

WHAT IS CLAIMED IS:

1. An expression system useful for the detection and isolation of a polypeptide capable of regulating a transduction pathway, the expression system comprising:

- (a) a first expression construct including a first coding region encoding a reporter molecule, said first coding region being under transcriptional control of a cis acting regulatory sequence element, said cis acting regulatory sequence element being regulatable by a trans acting regulator of the transduction pathway; and
- (b) an expression library including a plurality of second expression constructs, each of said plurality of second expression constructs of said expression library including a second coding region encoding for one of a plurality of polypeptides, said second coding region being under a transcriptional control of a promoter, such that when said first expression construct and a second expression construct of said plurality of second expression constructs of said

expression library are introduced into a cell, said cell endogenously expressing said trans acting regulator of the transduction pathway, a level of expression of said reporter molecule in said cell is indicative of regulation of the transduction pathway by a specific polypeptide of said plurality of polypeptides expressed by said cell from said second expression construct.

2. The expression system of claim 1, wherein said reporter molecule is an enzyme.

3. The expression system of claim 1, wherein said reporter molecule is a fluorescer.

4. The expression system of claim 3, wherein said fluorescer is selected from the group consisting of green fluorescent protein, blue fluorescent protein, yellow fluorescent protein and cyan fluorescent protein.

5. The expression system of claim 1, wherein said reporter molecule is a cell surface constituent.

6. The expression system of claim 1, wherein said first expression construct further includes a selectable marker sequence.

7. The expression system of claim 6, wherein said selectable marker sequence encodes a polypeptide capable of conferring antibiotic resistance to said cell.

8. The expression system of claim 1, wherein said second expression construct further includes a selectable marker sequence.

9. The expression system of claim 8, wherein said selectable marker sequence encodes a polypeptide capable of conferring antibiotic resistance to said cell when expressed therein.

10. The expression system of claim 8, wherein said selectable marker sequence encodes a fluorescer.

11. The expression construct of claim 1, wherein said cis acting regulatory sequence element is selected from the group consisting of a promoter, a transcriptional regulatory sequence and a translational regulatory sequence.

12. The expression system of claim 1, wherein said trans acting regulator of the transduction pathway is selected from the group consisting of a transcriptional regulator and a translational regulator.

13. The expression system of claim 1, wherein each of said plurality of second expression constructs of said expression library further includes a third coding region encoding a known polypeptide, said third coding region being translationally fused to said second coding region encoding for one of a plurality of polypeptides.

14. The expression system of claim 13, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides into a subcellular organelle.

15. The expression system of claim 14, wherein said subcellular organelle is a nucleus.

16. The expression system of claim 13, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides out of said cell.

17. The expression system of claim 1, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 5 amino acids to approximately 1000 amino acids.

18. The expression system of claim 17, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 10 amino acids to approximately 100 amino acids.

19. The expression system of claim 1, wherein said second coding region encoding for one of a plurality of polypeptides includes a polynucleotide sequence selected from the group consisting of a portion

of a polynucleotide sequence represented in a genome and a polynucleotide sequence not represented in a genome.

20. The expression system of claim 19, wherein said portion of a polynucleotide sequence represented in a genome is a digest product of a genome.

21. The expression system of claim 19, wherein said portion of a polynucleotide sequence represented in a genome is a PCR product.

22. The expression system of claim 1, wherein said polypeptide is selected from the group consisting of a characterized polypeptide, a portion of a characterized polypeptide, a combinatorial polypeptide and a polypeptide chimera.

23. The expression system of claim 1, wherein said cell is a eukaryotic cell.

24. An expression system useful for the detection and isolation of a polypeptide capable of regulating a transduction pathway, the expression system comprising:

- (a) a first expression construct including a first coding region encoding a transactivator, said first coding region being under transcriptional control of a cis acting regulatory sequence element, said cis acting regulatory sequence element being regulatable by a trans acting regulator of the transduction pathway; and
- (b) an expression library including a plurality of second expression constructs, each of said plurality of second expression constructs of said expression library including a second coding region encoding for one of a plurality of polypeptides, each of said plurality of second expression constructs of said expression library further including a third coding region encoding a reporter molecule, said second coding region and said third coding region being under a transcriptional control of at least one promoter being regulatable by said transactivator, such that when

said first expression construct and a second expression construct of said plurality of second expression constructs of said expression library are introduced into a cell, said cell endogenously expressing said trans acting regulator of the transduction pathway, a level of expression of said reporter molecule in said cell is indicative of regulation of the transduction pathway by a specific polypeptide of said plurality of polypeptides expressed by said cell from said second expression construct.

25. The expression system of claim 24, wherein said transactivator is selected from the group consisting of a transcriptional regulator and an RNA polymerase.

26. The expression system of claim 25, wherein said RNA polymerase is selected from the group consisting of a bacterial RNA polymerase and a bacteriophage RNA polymerase.

27. The expression system of claim 26, wherein said bacteriophage RNA polymerase is selected from the group consisting of a T7 RNA polymerase, a T3 RNA polymerase and an SP6 RNA polymerase.

28. The expression system of claim 24, wherein said reporter molecule is an enzyme.

29. The expression system of claim 24, wherein said reporter molecule is a fluorescer.

30. The expression system of claim 29, wherein said fluorescer is selected from the group consisting of green fluorescent protein, blue fluorescent protein, yellow fluorescent protein and cyan fluorescent protein.

31. The expression system of claim 24, wherein said reporter molecule is a eukaryotic cell surface marker.

32. The expression system of claim 24, wherein said first expression construct further includes a selectable marker sequence.

33. The expression system of claim 32, wherein said selectable marker sequence encodes a polypeptide capable of conferring antibiotic resistance to said cell.

34. The expression system of claim 24, wherein said second expression construct further includes a selectable marker sequence.

35. The expression system of claim 34, wherein said selectable marker sequence encodes a polypeptide capable of conferring antibiotic resistance to said cell.

36. The expression construct of claim 24, wherein said cis acting regulatory sequence element is selected from the group consisting of a promoter, a transcriptional regulatory sequence and a translational regulatory sequence.

37. The expression system of claim 24, wherein said transacting regulator of the transduction pathway is selected from the group consisting of a transcriptional regulator and a translational regulator.

38. The expression system of claim 24, wherein each of said plurality of second expression constructs of said expression library further includes a fourth coding region encoding a known polypeptide, said fourth coding region being translationally fused to said second coding region encoding for one of a plurality of polypeptides.

39. The expression system of claim 38, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides into a subcellular organelle.

40. The expression system of claim 39, wherein said subcellular organelle is a nucleus.

41. The expression system of claim 38, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides out of said cell.

42. The expression system of claim 24, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 5 amino acids to approximately 1000 amino acids.

43. The expression system of claim 42, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 10 amino acids to approximately 100 amino acids.

44. The expression system of claim 24, wherein said second coding region encoding for one of a plurality of polypeptides includes a polynucleotide sequence selected from the group consisting of a portion of a polynucleotide sequence represented in a genome and a polynucleotide sequence not represented in a genome.

45. The expression system of claim 44, wherein said portion of a polynucleotide sequence represented in a genome is a digest product of a genome.

46. The expression system of claim 44, wherein said portion of a polynucleotide sequence represented in a genome is a PCR product.

47. The expression system of claim 24, wherein said polypeptide is selected from the group consisting of a characterized polypeptide, a portion of a characterized polypeptide, a combinatorial polypeptide and a polypeptide chimera.

48. The expression system of claim 24, wherein said cell is a eukaryotic cell.

49. An expression library useful for the detection and isolation of a polypeptide capable of regulating a transduction pathway, the expression library comprising a plurality of expression constructs each including a first coding region encoding one polypeptide of a plurality of

polypeptides and a second coding region encoding a reporter molecule, said first and said second coding regions being under the transcriptional control of at least one cis acting regulatory sequence element being regulatable by a trans acting regulator of the transduction pathway, such that when an expression construct of said plurality of expression constructs of said expression library is introduced into a cell, said cell endogenously expressing said trans acting regulator of the transduction pathway, a level of expression of said reporter molecule in said cell is indicative of regulation of the transduction pathway by a specific polypeptide of said plurality of polypeptides expressed by said cell from said second expression construct.

50. The expression library of claim 49, wherein said reporter molecule is an enzyme.

51. The expression library of claim 49, wherein said reporter molecule is a fluorescer.

52. The expression library of claim 51, wherein said fluorescer is selected from the group consisting of green fluorescent protein, blue fluorescent protein, yellow fluorescent protein and cyan fluorescent protein.

53. The expression library of claim 49, wherein said reporter molecule is a eukaryotic cell surface marker.

54. The expression library of claim 49, wherein each of said plurality of expression constructs further includes a selectable marker sequence.

55. The expression library of claim 54, wherein said selectable marker sequence encodes a polypeptide capable of conferring antibiotic resistance to said cell.

56. The expression library of claim 49, wherein said cis acting regulatory sequence element is selected from the group consisting of a

promoter, a transcriptional regulatory sequence and a translational regulatory sequence.

57. The expression library of claim 49, wherein said transacting regulator of the transduction pathway is selected from the group consisting of a transcriptional regulator and a translational regulator.

58. The expression library of claim 49, wherein each of said plurality of expression constructs further includes a third coding region encoding a known polypeptide, said third coding region being translationally fused to said first coding region encoding for one of a plurality of polypeptides.

59. The expression library of claim 58, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides into a subcellular organelle.

60. The expression library of claim 59, wherein said subcellular organelle is a nucleus.

61. The expression library of claim 58, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides out of said cell.

62. The expression library of claim 49, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 5 amino acids to approximately 1000 amino acids.

63. The expression library of claim 62, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 10 amino acids to approximately 100 amino acids.

64. The expression library of claim 49, wherein said first coding region encoding for one of a plurality of polypeptides includes a polynucleotide sequence selected from the group consisting of a portion of a polynucleotide sequence represented in a genome and a polynucleotide sequence not represented in a genome.

65. The expression library of claim 64, wherein said portion of a polynucleotide sequence represented in a genome is a digest product of a genome.

66. The expression library of claim 64, wherein said portion of a polynucleotide sequence represented in a genome is a PCR product.

67. The expression library of claim 49, wherein said polypeptide is selected from the group consisting of a characterized polypeptide, a portion of a characterized polypeptide, a combinatorial polypeptide and a polypeptide chimera.

68. The expression library of claim 49, wherein said cell is a eukaryotic cell.

69. A method of detecting a polypeptide capable of regulating a transduction pathway, the method comprising the step of:

- (a) introducing into cells endogenously expressing a transacting regulator of the transduction pathway a first

expression construct, said first expression construct including a first coding region encoding a reporter molecule, said first coding region being under transcriptional control of a cis acting regulatory sequence element, said cis acting regulatory sequence element being regulatable by said trans acting regulator of the transduction pathway; and

- (b) introducing into at least a portion of said cells an expression library, said expression library including a plurality of second expression constructs, each of said plurality of second expression constructs of said expression library including a second coding region encoding for one of a plurality of polypeptides, said second coding region being under a transcriptional control of a promoter,
- (c) monitoring a level of expression of said reporter molecule in said cells, said level of expression within a predetermined range being indicative of regulation of the transduction pathway by a polypeptide of said plurality of polypeptides; and

- (d) isolating said second coding region from a cell of said cells
in which said level of expression of said reporter molecule
is within said predetermined range.

70. The method of claim 69, wherein said reporter molecule is
an enzyme.

71. The method of claim 69, wherein said reporter molecule is
a fluorescer.

72. The method of claim 71, wherein said fluorescer is selected
from the group consisting of green fluorescent protein, blue fluorescent
protein, yellow fluorescent protein and cyan fluorescent protein.

73. The method of claim 69, wherein said reporter molecule is
a eukaryotic cell surface marker.

74. The method of claim 69, wherein steps (a) and (b) are each
effected via a transformation method selected from the group consisting

of biolistic bombardment, direct DNA uptake, virus mediated transformation and calcium phosphate transformation.

75. The method of claim 69, wherein steps (a) and (b) are co-effected via a single step.

76. The method of claim 69, further including a step of selecting for cells expressing said reporter molecule prior to said introducing of said expression library.

77. The method of claim 69, wherein said step of monitoring a level of expression of said reporter molecule in said cells is effected via an automated cell sorter.

78. The method of claim 69, wherein said step of isolating said second coding region is effected via a PCR reaction using oligonucleotide primers flanking said second coding region.

79. The method of claim 69, wherein said cis acting regulatory sequence element is selected from the group consisting of a promoter, a transcriptional regulatory sequence and a translational regulatory sequence.

80. The method of claim 69, wherein said trans acting regulator of the transduction pathway is selected from the group consisting of a transcriptional regulator and a translational regulator.

81. The method of claim 69, wherein each of said plurality of second expression constructs of said expression library further includes a third coding region encoding a known polypeptide, said third coding region being translationally fused to said second coding region encoding for one of a plurality of polypeptides.

82. The method of claim 81, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides into a subcellular organelle.

83. The method of claim 82, wherein said subcellular organelle is a nucleus.

84. The method of claim 81, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides out of said cell.

85. The method of claim 69, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 5 amino acids to approximately 1000 amino acids.

86. The method of claim 85, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 10 amino acids to approximately 100 amino acids.

87. The method of claim 69, wherein said second coding region encoding for one of a plurality of polypeptides includes a polynucleotide sequence selected from the group consisting of a portion

of a polynucleotide sequence represented in a genome and a polynucleotide sequence not represented in a genome.

88. The method of claim 87, wherein said portion of a polynucleotide sequence represented in a genome is a digest product of a genome.

89. The method of claim 87, wherein said portion of a polynucleotide sequence represented in a genome is a PCR product.

90. The method of claim 69, wherein said polypeptide is selected from the group consisting of a characterized polypeptide, a portion of a characterized polypeptide, a combinatorial polypeptide and a polypeptide chimera.

91. The method of claim 69, wherein said cell is a eukaryotic cell.

92. A method of detecting a polypeptide capable of regulating a transduction pathway, the method comprising the step of:

- (a) introducing into cells endogenously expressing a transacting regulator of the transduction pathway a first expression construct, said first expression construct including a first coding region encoding a transactivator, said first coding region being under transcriptional control of a cis acting regulatory sequence element, said cis acting regulatory sequence element being regulatable by said transacting regulator of the transduction pathway; and
- (b) introducing into at least a portion of said cells an expression library including a plurality of second expression constructs, each of said plurality of second expression constructs of said expression library including a second coding region encoding for one of a plurality of polypeptides, each of said plurality of second expression constructs of said expression library further including a third coding region encoding a reporter molecule, said second coding region and said third coding region being

under a transcriptional control of at least one promoter being regulatable by said transactivator,

- (c) monitoring a level of expression of said reporter molecule in said cells, said level of expression within a predetermined range being indicative of regulation of the transduction pathway by a polypeptide of said plurality of polypeptides; and
- (d) isolating said second coding region from a cell of said cells in which said level of expression of said reporter molecule is within said predetermined range.

93. The method of claim 92, wherein said transactivator is selected from the group consisting of a transcriptional regulator and an RNA polymerase.

94. The method of claim 93, wherein said RNA polymerase is selected from the group consisting of a bacterial RNA polymerase and a bacteriophage RNA polymerase.

95. The method of claim 94, wherein said bacteriophage polymerase is selected from the group consisting of a T7 RNA polymerase, a T3 RNA polymerase and an SP6 RNA polymerase.

96. The method of claim 92, wherein steps (a) and (b) are each effected via a transformation method selected from the group consisting of biolistic bombardment, direct DNA uptake, virus mediated transformation and calcium phosphate transformation.

97. The method of claim 92, wherein steps (a) and (b) are co-effected via a single step.

98. The method of claim 92, further including a step of selecting for cells expressing said reporter molecule prior to said introducing of said expression library.

99. The method of claim 92, wherein said step of monitoring a level of expression of said reporter molecule in said cells is effected via an automated cell sorter.

100. The method of claim 92, wherein said step of isolating said second coding region is effected via a PCR reaction using oligonucleotide primers flanking said second coding region.

101. The method of claim 92, wherein said cis acting regulatory sequence element is selected from the group consisting of a promoter, a transcriptional regulatory sequence and a translational regulatory sequence.

102. The method of claim 92, wherein said trans acting regulator of the transduction pathway is selected from the group consisting of a transcriptional regulator and a translational regulator.

103. The method of claim 92, wherein each of said plurality of second expression constructs of said expression library further includes a fourth coding region encoding a known polypeptide, said fourth coding region being translationally fused to said second coding region encoding for one of a plurality of polypeptides.

104. The method of claim 103, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides into a subcellular organelle.

105. The method of claim 104, wherein said subcellular organelle is a nucleus.

106. The method of claim 92, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides out of said cell.

107. The method of claim 92, wherein each of said plurality of polypeptides is of a specific size selected from size range of approximately 5 amino acids to approximately 1000 amino acids.

108. The method of claim 107, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 10 amino acids to approximately 100 amino acids.

109. The method of claim 92, wherein said second coding region encoding for one of a plurality of polypeptides includes a polynucleotide sequence selected from the group consisting of a portion of a polynucleotide sequence represented in a genome and a polynucleotide sequence not represented in a genome.

110. The method of claim 109, wherein said portion of a polynucleotide sequence represented in a genome is a digest product of a genome.

111. The method of claim 109, wherein said portion of a polynucleotide sequence represented in a genome is a PCR product.

112. The method of claim 92, wherein said polypeptide is selected from the group consisting of a characterized polypeptide, a portion of a characterized polypeptide, a combinatorial polypeptide and a polypeptide chimera.

113. The method of claim 92, wherein said cell is a eukaryotic cell.

114. A method of detecting a polypeptide capable of regulating a transduction pathway, the method comprising the step of:

- (a) introducing into cells endogenously expressing a trans acting regulator of the transduction pathway an expression library, said expression library including a plurality of expression constructs each including a first coding region encoding one polypeptide of a plurality of polypeptides and a second coding region encoding a reporter molecule, said first and said second coding regions being under the transcriptional control of at least one cis acting regulatory sequence element being regulatable by said trans acting regulator of the transduction pathway,
- (b) monitoring the level of expression of said reporter molecule in said cells, a level of expression within a predetermined range being indicative of regulation of the transduction

pathway by a polypeptide of said plurality of polypeptides;

and

- (c) isolating said first coding region from a cell of said cells in which the level of expression of said reporter molecule is within said predetermined range.

115. The method of claim 114, wherein said reporter molecule is an enzyme.

116. The method of claim 114, wherein said reporter molecule is a fluorescer.

117. The method of claim 116, wherein said fluorescer is selected from the group consisting of green fluorescent protein, blue fluorescent protein, yellow fluorescent protein and cyan fluorescent protein.

118. The method of claim 114, wherein said reporter molecule is a eukaryotic cell surface marker.

119. The method of claim 114, wherein step (a) is effected via a transformation method selected from the group consisting of biolistic bombardment, direct DNA uptake, virus mediated transformation and calcium phosphate transformation.

120. The method of claim 114, wherein said step of monitoring a level of expression of said reporter molecule in said cells is effected via an automated cell sorter.

121. The method of claim 114, wherein said step of isolating said first coding region is effected via a PCR reaction using oligonucleotide primers flanking said first coding region.

122. The method of claim 114, wherein said cis acting regulatory sequence element is selected from the group consisting of a promoter, a transcriptional regulatory sequence and a translational regulatory sequence.

123. The method of claim 114, wherein said trans acting regulator of the transduction pathway is selected from the group consisting of a transcriptional regulator and a translational regulator.

124. The method of claim 114, wherein each of said plurality of expression constructs further includes a third coding region encoding a known polypeptide, said third coding region being translationally fused to said first coding region encoding for one of a plurality of polypeptides.

125. The method of claim 124, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides into a subcellular organelle.

126. The method of claim 125, wherein said subcellular organelle is a nucleus.

127. The method of claim 124, wherein said known polypeptide is capable of targeting said one of a plurality of polypeptides out of said cell.

128. The method of claim 114, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 5 amino acids to approximately 1000 amino acids.

129. The method of claim 128, wherein each of said plurality of polypeptides is of a specific size selected from a size range of approximately 10 amino acids to approximately 100 amino acids.

130. The method of claim 114, wherein said first coding region encoding for one of a plurality of polypeptides includes a polynucleotide sequence selected from the group consisting of a portion of a polynucleotide sequence represented in a genome and a polynucleotide sequence not represented in a genome.

131. The method of claim 130, wherein said portion of a polynucleotide sequence represented in a genome is a digest product of a genome.

132. The method of claim 130, wherein said portion of a polynucleotide sequence represented in a genome is a PCR product.

133. The method of claim 114, wherein said polypeptide is selected from the group consisting of a characterized polypeptide, a portion of a characterized polypeptide, a combinatorial polypeptide and a polypeptide chimera.

134. The method of claim 114, wherein said cell is a eukaryotic cell.